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DRUG RETENTION DURING ANIMAL INHALATION  
EXPOSURE BY FT-IR SPECTROSCOPY

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JUL 27 1990  
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May 1990

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## FOREWORD

The work described in this report was directly related to research authorized under US Army Medical Research Institute of Chemical Defense (USAMRICD) protocol number 1-03-87-000-A-422 and by JSA requirements C-A-306, C-A-309, and C-A-325. The original research data were recorded in USAMRICD laboratory notebook number 033-88. The authors wish to thank MAJ Kenneth G. Phillips for assisting in the design of the inhalation exposure system, Dr. Holcombe H. Hurt, Jr. for administering the anesthesia and intubation of the trachea, and SGT Felix Feliciano-Emmanuelli for his assistance in setting up the inhalation exposure system.

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## INTRODUCTION

To determine the role of pulmonary edema in the pathophysiology of pulmonary irritants, the Analytical Chemistry Branch of the US Army Medical Research Institute of Chemical Defense (USAMRIOD) was tasked to develop an analytical method to determine the amount of drug retained by animals during inhalation exposure experiments. In addition, the method was required to determine whether decomposition of the test compound occurred during animal exposure.

The ability of IR spectroscopy to quantify ppm (part per million) concentrations of components in a gaseous mixture, coupled with its ability to qualitatively identify unknown components, made the use of this technique a logical choice for achieving the stated task. In this report, we describe an analytical method for determining the amount of drug retained by animals during inhalation exposure experiments. We selected bis-(trifluoromethyl) disulfide (TFD), a commercially available (1) toxic compound often encountered in industry and agriculture, as a test compound.

## MATERIALS AND METHODS

**Equipment and Supplies.** A Nicolet 5SX-B FT-IR spectrometer utilizing a liquid nitrogen-cooled mercury cadmium telluride (MCT-A) detector, a germanium-on-KBr beam splitter, and air cooled globar source were used to record all spectra. The optical retardation velocity was 0.198 cm/sec, and the laser frequency was 3.13 kHz. Instrumental parameters were optimized to give the best results and are listed in Table I. The sample compartment was open to the room atmosphere, and the instrument was not purged. Spectra recorded were the result of 32 co-added interferograms at 4.0  $\text{cm}^{-1}$  resolution. Happ-Genzel apodization was used in the subsequent Fourier transform (FT).

Table I. FT-IR Parameters

Parameter	Value	Parameter	Value
AFN	HG	APT	FL
AXS	YS	BDL	15
BNF	3	CBM	NO
COR	Lo	CXF	-500
CYT	25.00	FIT	YES
FSZ	22528	FXF	4000
FYT	0.0	GAN	4
HPS	0	LSP	0
LXF	500.0	LYT	125.0
MIR	SB	NDP	6144
NPD	200	NPT	1024
NSB	32	NSD	32
NSS	32	NSK	0
NTP	8192	PAG	YS
PHZ	PH	PLO	100
SBM	FT	SFN	2
SGH	0	SGL	0
SSP	2	VEL	40
XEP	500	XSP	4000
YEP	2.50	YSL	5
YSP	4000		

All spectra were run using a variable long pathlength gas cell (Barnes Analytical Division, Spectra Tech Inc., 652 Glenbrook Road, Stamford, CT 06906). The gas cell had a volume of 2.5 L and was constructed of gold-coated cast aluminum. All experiments used KBr windows and a pathlength of 10 meters.

Figure 1 gives a block diagram of the experimental set-up during the collection phase of the experiment, and Figure 2 gives a block diagram of the experimental set-up for the analysis phase of the experiment. Figure 3 gives a block diagram of the apparatus used to concentrate the collected products, and Figure 4 shows the equipment used to analyze the concentrated products.

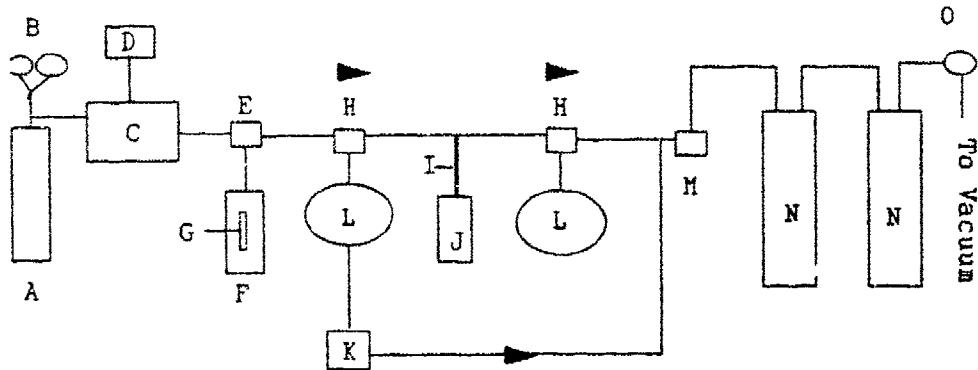


Figure 1. Collection System. A - compressed air tank, B - dual stage regulator, C - mass flow controller, model FC-261, Tytan Corp., D - mass flow controller module, model RO-28, Tyler Corp., E - injection port (butyl rubber), F - syringe pump, model 335, Sage Instruments, G - gas tight Hamilton syringe, 1000  $\mu$ l, model 1001, H - Hans Rudolph valve, I - endo-tracheal tube (used with animal), J - animal or respirator pump, model 613, Harvard Apparatus, K - air circulating pump, model MB-41, Metal Bellows, L - inhalation reservoir bag (4 L), M - one-way valve, N - plastic drum (208 L), O - shut-off valve.

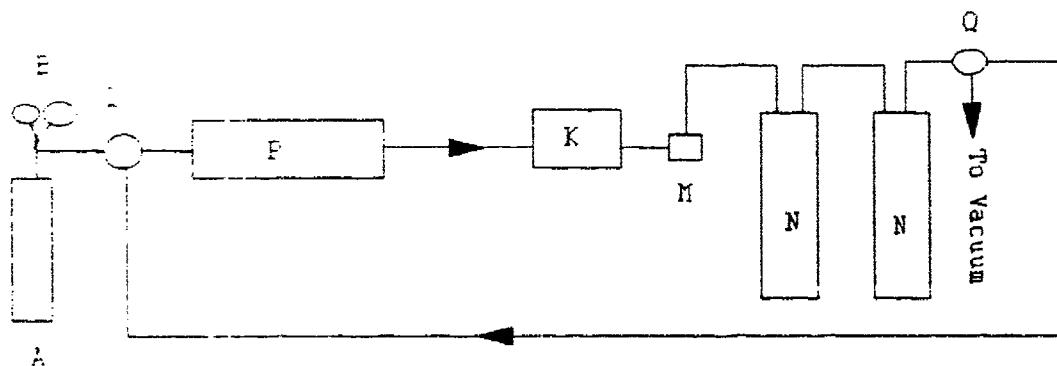


Figure 2. Analysis System. A - compressed air tank, B - dual stage regulator, K - air circulating pump, model MB-41, Metal Bellows, M - one-way valve, N - plastic drum (208 L), P - FT-IR gas cell, Q - 3-way valve.

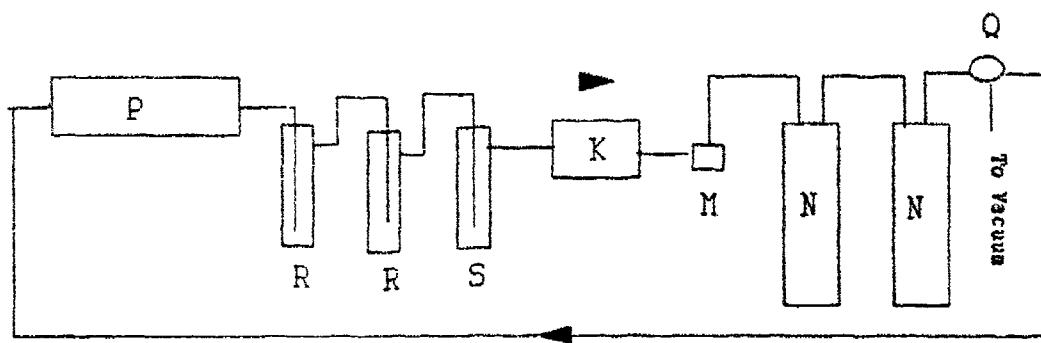


Figure 3. Concentration System. K - air circulating pump, model MB-41, Metal Bellows, M - one-way valve, N - plastic drum (208 L), P - FT-IR gas cell, Q - 3-way valve, R - dry-ice cold trap, S - liquid nitrogen cold trap.

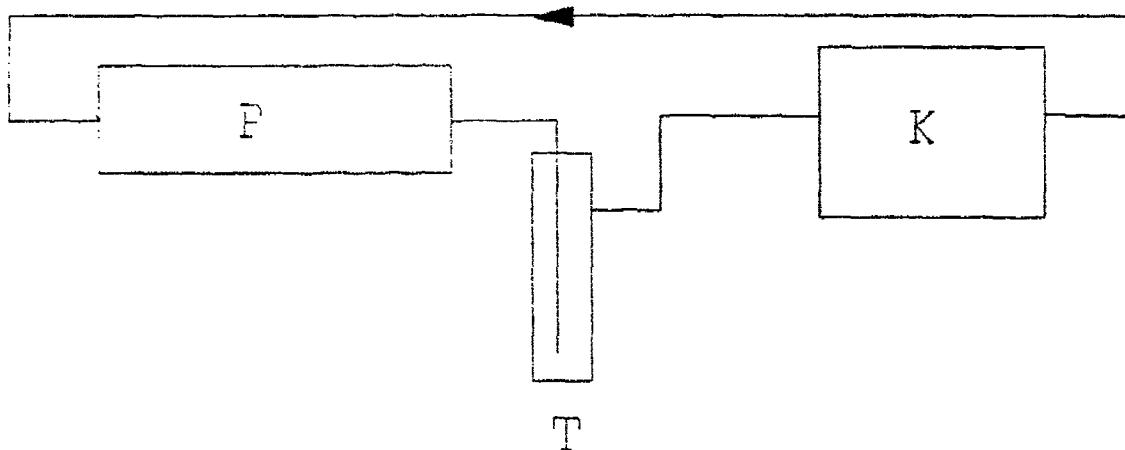


Figure 4. Concentration System for Analysis. K - air circulating pump, model MB-41, Metal Bellows, P - FT-IR gas cell, T - cold trap at room temperature.

**Procedure.** In preparation for the animal exposure, the analysis system described in Figure 2 was flushed completely with the compressed air. A spectrum was recorded, stored to hard disk on the FT-IR spectrometer, and used as the background spectrum in the analysis. The collection system, which included part of the analysis system, was evacuated using house vacuum in order to collapse the 208 liter plastic drums. The collection system, however, was at room pressure at the start of collecting the sheep's expired gas. Before connecting the sheep to the collection system a sample of the sheep's expired gas was collected in the FT-IR gas cell and the spectrum recorded. A ratio of this spectrum with the background spectrum was made and stored to hard disk. The ratioed spectrum of the normal sheep's expired gas

was used as the reference spectrum for spectral subtraction during quantitative analysis. The sheep or respirator pump was connected to the collection system as described in Figure 1. The expired gas from the sheep and the air through the by-pass loop were completely collected into the collapsed plastic tanks. An inhalation reservoir bag and air pump on the by-pass loop were manually controlled by an operator to ensure that a positive pressure did not force air into the animal. A second inhalation reservoir bag allowed the animal to exhale easily. An operator manually collapsed this bag during animal inhalation, forcing the exhaled air into the storage tanks. A 1000  $\mu$ l Hamilton gas-tight syringe was filled with TFD (PCR Inc, P.O. Box 1466, Gainesville, FL 32602, Cat No. 185009, CAS No 372-64-5) and the beginning volume recorded. The syringe was placed into the syringe pump, the needle inserted into the septum port and the syringe pump started. The animals were exposed to a concentration of 5.0 mg compound per liter of air for 10 minutes; then the syringe was removed and the amount of remaining liquid noted. The amount of compound presented to the animal was determined from the initial and final reading of the syringe. The ability to read the volume dispensed by the syringe pump accurately was confirmed by simulating the syringe pump injection procedure using water in the syringe and quantitatively weighing the water dispensed.

The sheep used in this study was maintained using standard large animal procedures with supporting veterinary care. No premedication was used. In preparation for TFD exposure the sheep was placed in a metabolism cage and supported by means of a fish net sling adjusted to support nearly its full weight. A balloon tipped flow directed catheter was used to administer a 10 mg/ml solution of ketamine in normal saline into the pulmonary artery. The ketamine was given in increments, in amounts sufficient to produce unconsciousness of sufficient relaxation to permit oral intubation of the trachea. Thereafter, additional amounts were given at roughly 5-min intervals by the same route to maintain a level of anesthesia adequate to allow the sheep to tolerate the exposure to TFD and the continuing intubation while vital signs were monitored. At 4 hours post-TFD exposure the sheep was given pentobarbital (50 mg/kg) intravenously (IV) to achieve a surgical plane of anesthesia. The sheep was then exsanguinated by bilateral sectioning of the jugular veins and carotid arteries.

After the 10-min exposure period, the animal remained connected to the collection system until both collecting tanks were completely filled. This typically took about 25 min with an air flow rate of 15 liters per min. After the tanks were completely filled the animal was disconnected from the collecting system, and a sample of the animal's expired gas was directed to the FT-IR gas cell for analysis. The recorded spectrum confirmed that the animal was not exhaling any of the tested compound at this time.

The one-way valve before the first collecting tank (Figure 1) prevented loss of test compound when the collecting system was switched to the analysis system. Before analysis of the test compound its concentration was allowed to come to equilibrium. The equilibration process took about 2 hours using the air circulating pump. Equilibrium was confirmed by observing a constant IR absorbance value for the test compound. The concentration of the test compound was determined by the average absorbance value of three consecutive IR spectra.

Quantification of TFD used the absorbance band at 758 cm<sup>-1</sup>. Standard Nicolet software allowed for the spectral subtraction of the reference sheep breath. The FCR subtraction factor was visually determined for each spectrum and recorded. After manual baseline correction, the maximum peak height (absorbance) of the 758 cm<sup>-1</sup> band was determined by the PPK Nicolet command.

The concentration of TFD was determined from the calibration experiment and the Beer's law relationship. For the calibration experiment a control sheep was used in the standard apparatus, but TFD was not injected into the system. After filling the collection system (Figure 1) the apparatus was switched to the analysis system (Figure 2). A volume of 500  $\mu$ l of TFD was injected into the analysis loop using the 1000  $\mu$ l Hamilton gas tight syringe, and the air was circulated using the air circulation pump. When no change was observed in the absorbance of the 758 cm<sup>-1</sup> band, the maximum peak height of this absorbance band was determined as described above.

After the concentration of TFD was determined in the analysis system of Figure 2, the collected gas from the animal was concentrated using the apparatus described in Figure 3. The collected gas was circulated for at least 12 hours allowing the organic gases to be collected in the cold traps. After the collection period, each of the cold traps was analyzed by IR spectroscopy using the apparatus described in Figure 4.

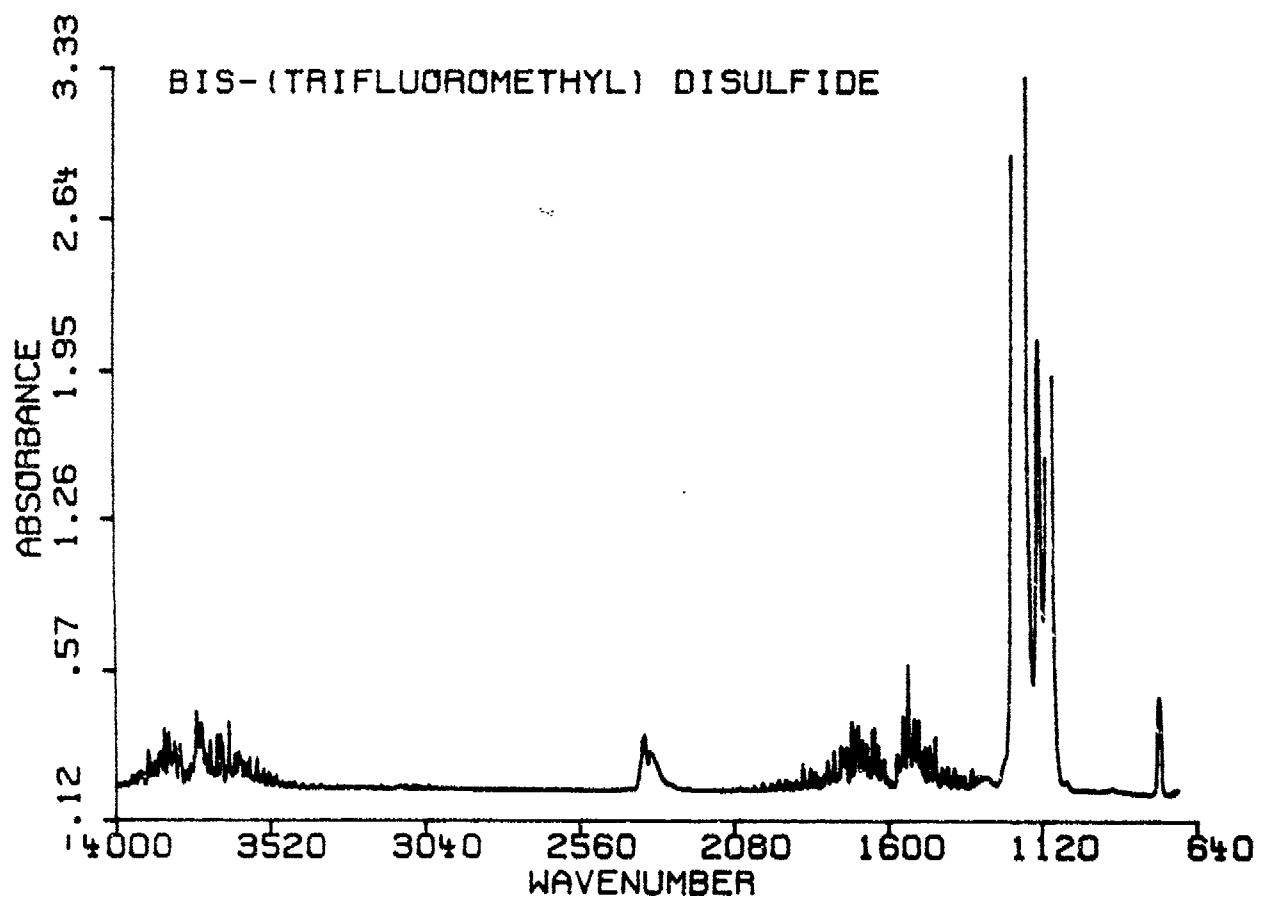
#### RESULTS AND DISCUSSION

The IR spectrum of TFD (Figure 5) was recorded after 290  $\mu$ l was injected into the Analysis system (Figure 2). The C-F stretching bands at 1101, 1133, 1189, and 1204 cm<sup>-1</sup> are all off scale. The C-S stretching absorption band at 758 cm<sup>-1</sup> was chosen as the quantitative peak because of its location and extinction coefficient. The relatively small peak at 758 cm<sup>-1</sup> was clear of interferences and gave an almost ideal quantitative absorbance value of 0.81 a.u. (absorbance units) for a 500  $\mu$ l injection. The IR spectrum of the exhaled sheep breath showed several absorption bands, but these were effectively subtracted out using the standard Nicolet software during quantification of TFD.

The total volume of the analysis system was estimated to be 420 liters. After injection of a 500  $\mu$ l sample into the analysis system, equilibrium was achieved after 1 to 2 hours, which was confirmed by the observation of a constant absorbance value for the 758 cm<sup>-1</sup> peak.

The analysis system was calibrated by injection of 500  $\mu$ l of TFD. Analysis in triplicate of the absorbance values for the 758 cm<sup>-1</sup> peak gave a mean value of 0.810 a.u., with a standard deviation of 0.0106 ( $n=3$ ) and a

Figure 5. IR Spectrum of Bis-(trifluoromethyl) disulfide, 290  $\mu$ l in 420 L, 10 m pathlength.



coefficient of variation (CV) 1.3 %. The Beer's law relationship  $A = abc$  where

A = absorbance  
a = molar absorptivity constant  
b = pathlength  
c = concentration

was used for the quantification. Since the terms "a" and "b" as well as the total volume of the system were all constant for these experiments, Beer's law was reduced to  $A = Kv$  where

K = grouping of all constants  
v = volume of sample compound in the system

The K value for these experiments was calculated from the calibration experiment to be  $1.62 (\pm 0.02) \times 10^3$ . This K value was used to calculate the amount of TFD in the other experiments.

Although a single point calibration was used for the sheep exposure and control study, good linearity for the analysis of TFD by this technique was established in a modified experimental setup. The apparatus setup used was similar to that described in Figure 4, except that the total volume was about 8 liters. Using this 8-liter system, the analytical data obtained is summarized in Table II.

Table II. Calibration Data

Volume, $\mu\text{l}$	Mean	SD (n=3)	% C.V.*	Mean
	TFD			Absorbance
		758 cm <sup>-1</sup>		1101 cm <sup>-1</sup>
1.00	0.0627	0.00382	6.09	0.852
2.00	0.122	0.00115	0.94	1.77
3.00	0.182	0.00551	3.03	off scale

\* Percent coefficient of variation,  $(\text{SD}/\text{mean})100$

The Beer's law plot for this data is given in Figure 6. The correlation coefficient was calculated to be 1.000. As can be observed, the linearity is excellent.

A control experiment was performed using a respirator pump instead of a sheep (Table III). All other experimental conditions were the same as the actual sheep exposure. In this experiment, 290  $\mu\text{l}$  of TFD was injected during the 10-min exposure period. Within experimental error, all of the injected sample was recovered in the collection system. This control experiment demonstrated the collection system's ability to determine whether injected sample was retained by the animal.

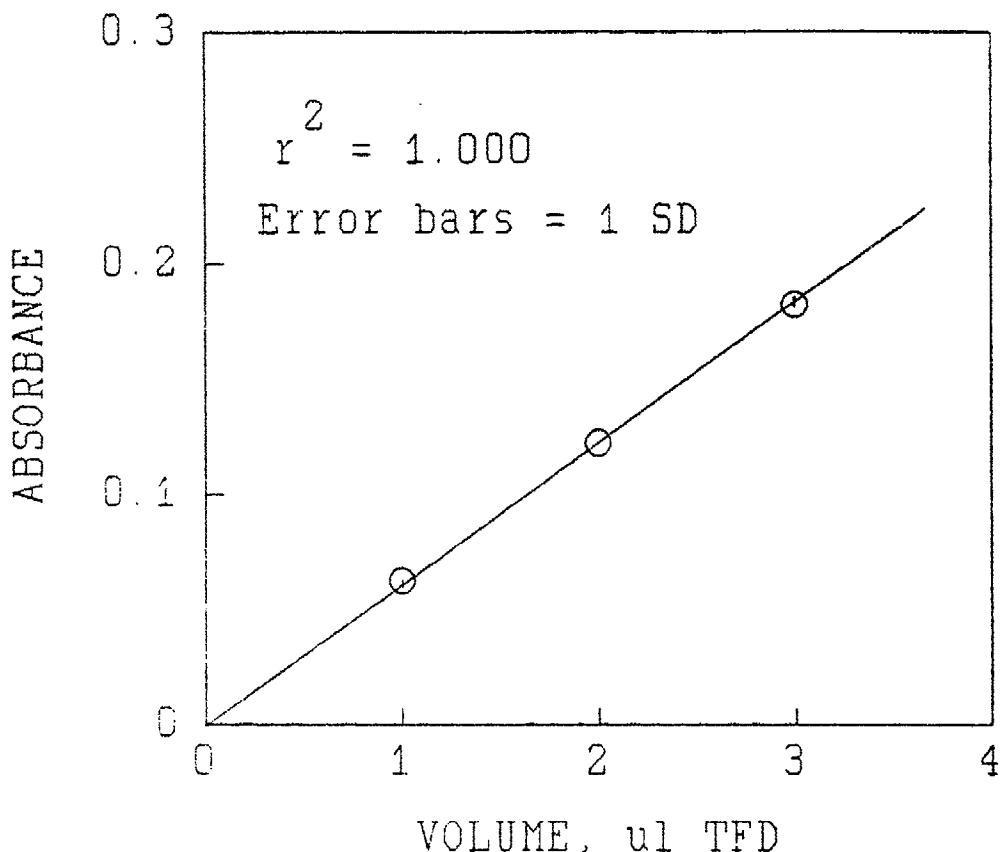


Figure 6. Beer's law working curve for calibration data. Volume of neat TFD injected into constant volume gas cell with 10 m pathlength.

Table III. Experimental Data

EXPERIMENT	MEAN ABSORBANCE $758 \text{ cm}^{-1}$	SD (N=3)	CALC TFD $\mu\text{l}$	INJECTED TFD $\mu\text{l}$	%LOSS TFD
Calibration	0.810	0.0106	----	500	----
Exposure	0.474	0.0016	292 ( $\pm 4$ )	425	31%
Control	0.459	0.0037	283 ( $\pm 4$ )	290	2%

Data from the sheep exposure experiment are summarized in Table III. The sheep used in the exposure weighed 44.5 Kg, and a total of  $1.3 \times 10^2 \mu\text{l}$  of TFD was retained by the sheep. This represents a retention of TFD, by the sheep, of  $2.9 \mu\text{l}$  (4.4 mg) per Kg of body weight (density of TFD equals 1.5 mg/ml).

Another goal of this study was to determine whether the compound to be evaluated would undergo any chemical change during the animal exposure period. An expected decomposition product of TFD is trifluoromethyl sulfide. The S-H stretching band for trifluoromethyl sulfide should be located in the 2590-2550 cm<sup>-1</sup> range.\* An absorbance band in this range was not observed for the products trapped in the collection system from either the control or the sheep exposure experiments. Due to the large volume (420 L) of the analysis system (Figure 2) small amounts of the decomposition products would most likely be below the detection limit of analysis technique. Figure 3 describes an apparatus system used to concentrate the collected gases. It was observed that -78 deg C (dry ice temperature) was not cold enough to trap out TFD (boiling point, 34-35 deg C). A liquid nitrogen (-196 deg C) cold trap was successful. Due to the large amount of water vapor and carbon dioxide exhaled by the animal, a series of three traps was necessary to effectively trap TFD. The first two dry-ice cold traps removed enough water so that the liquid nitrogen cold trap did not become clogged.

IR analysis (Figure 4) of the concentrated products from the sheep exposure and control experiments did not yield an S-H absorbance band. This indicates TFD did not decompose to form trifluoromethyl sulfide.

#### CONCLUSIONS

It was demonstrated that the analytical method developed using a large-volume collection system, a long pathlength gas cell, and FT-IR spectroscopy is well suited for determining the amount of drug retained by animals during inhalation exposure experiments. The analytical method can also be used to determine whether any decomposition products of the tested compound are exhaled by the animal.

It was observed that TFD is retained in sheep during inhalation exposure. The amount of retention in sheep was observed to be 4.4 mg TFD per Kg of body weight following a dose of 14.3 mg per Kg of body weight.

We did not observe TFD to undergo chemical decomposition during the exposure period.

We recommend this analytical method for evaluation of the amount of drug retained by animals during inhalation exposure experiments.

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\*L. J. Bellamy, The Infrared Spectra of Complex Molecules (Chapman and Hall Ltd, London, Eng, 1975), 3rd ed., pp 395-397.

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